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The dopamine D_4 Receptor (DRD4) gene exon III polymorphism, problematic alcohol use and novelty seeking: direct and mediated genetic effects

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ABSTRACT

The present study sought to integrate convergent lines of research on the associations among the dopamine D_4 receptor (DRD4) gene, novelty seeking and drinking behaviors with the overall goal of elucidating genetic influences on problematic drinking in young adulthood. Specifically, this study tested a model in which novelty seeking mediated the relationship between DRD4 variable number of tandem repeats (VNTR) genotype and problematic alcohol use. Participants (n = 90, 40 females) were heavy-drinking college students. Analyses using a structural equation modeling framework suggested that the significant direct path between DRD4 VNTR genotype and problematic alcohol use was reduced to a trend level in the context of a model that included novelty seeking as a mediator, thereby suggesting that the effects of DRD4 VNTR genotype on problematic alcohol use among heavy-drinking young adults were partially mediated by novelty seeking. Cross-group comparisons indicated that the relationships among the model variables were not significantly different in models for men versus women. These results extend recent findings of the association between this polymorphism of the DRD4 receptor gene, problematic alcohol use and novelty seeking. These findings may also help elucidate the specific pathways of risk associated with genetic influences on alcohol use and abuse phenotypes.

Keywords alcohol, DRD4 VNTR, genetics, mediation, novelty seeking, structural equation modeling.

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INTRODUCTION

Genetic variation in neurotransmitter systems has been implicated in multiple behavioral phenotypes and psychiatric disorders. The dopamine D_4 receptor (DRD4) has been examined as a gene of interest for behavioral and psychiatric phenotypes in part because of its theoretical plausibility and genetic variability. The DRD4 gene contains a 48-base pair variable number of tandem repeats (VNTR) in exon III with lengths varying from two to 11 repeats, three with common variants of 2 (D4.2), 4 (D4.4) and 7 repeats (D4.7) (Van Tol *et al.*, 1992; Grady *et al.*, 2003). Variations in length of the VNTR have been shown to have functional effects on the receptor (Asghari *et al.*, 1995; Oak, Oldenhof & Van Tol 2000). In vitro, while the D4.7 variant does not appear to bind dopamine antagonists and agonists with greater affinity than the D4.2 or D4.4 variants (Van Tol *et al.*, 1992; Asghari *et al.*, 1994), there is evidence that the D4.7 demonstrates a blunted intracellular response to dopamine (Asghari *et al.*, 1995). This relationship is such that the D4.7 variant is associated with attenuated inhibition of intracellular cyclic AMP (Oak *et al.*, 2000). It should be noted that the VNTR is found in the third cytoplasmic loop of the folded protein, which interacts with SH3 domain-binding proteins but does not appear to be a G-protein coupling site (Asghari *et al.*, 1994; Oak *et al.*, 2000).

D_4 receptors are structurally very similar to D_2 receptors and are localized in various brain regions, including the cerebral cortex, amygdala, hypothalamus, the pituitary and other limbic brain structures (Van Tol *et al.*, 1991; Asghari *et al.*, 1995). Expression of D_4 receptors in the prefrontal cortex is of particular interest for behavioral phenotypes as these regions are involved in attention and cognition (Oak *et al.*, 2000). Results from the animal literature suggest that DRD4 knockout mice display hypersensitivity to drugs of abuse such as ethanol, cocaine and methamphetamine (Rubinstein *et al.*, 1997). Other studies using DRD4 knockout mice show they perform better than their wild-type litter mates on complex motor tasks (Rubinstein *et al.*, 1997) and show enhanced cortical glutamate neuronal activity (Rubinstein *et al.*, 2001), supporting the notion that DRD4 receptors normally act

as inhibitors of neuronal activity. Interestingly, DRD4 knockout mice show decreased behavioral exploration of novel stimuli (Dulawa *et al.*, 1999).

In the human literature, genetic variation in the DRD4 gene has been examined in the context of personality traits [i.e. novelty seeking (NS) and impulsivity] and alcohol-related (i.e. drinking and alcohol craving) phenotypes, among other areas. NS has been conceptualized as a dopamine-mediated heritable tendency toward exploration and excitement in response to novel stimuli (Cloninger, Svrakic & Przybeck 1993). As such, genetic variation in the DRD4 VNTR represents a plausible candidate gene underlying NS behavior. Research on the association between the DRD4 VNTR genotype and NS has produced conflicting results. Some studies have found support for an association between the DRD4 VNTR and NS, such that carriers of the 7 repeat 'long' (L; i.e. $\geq$7 repeats) allele of the DRD4 VNTR score higher on measures of NS as compared with non-carriers (e.g. Laucht *et al.*, 2005, 2007). However, other studies have failed to replicate these findings (for a review see Kluger, Siegfried & Ebstein 2002).

In the context of alcohol-related phenotypes, the DRD4 VNTR has also produced equivocal findings. Although the direct association between DRD4 and alcohol diagnosis has yielded largely negative results (Tyndale 2003), the DRD4 VNTR has been significantly associated with alcohol craving in the laboratory, such that carriers of an L allele exhibited higher alcohol craving and consumption, as compared with individuals who were homozygotes for the 'short (S) allele' (i.e. <7 repeats) (Hutchison *et al.* 2002, 2003, 2006; McGeary *et al.* 2006; MacKillop *et al.* 2007), although a recent study failed to replicate these findings (van den Wildenberg *et al.* 2007). NS and drinking behaviors have in turn been associated in the literature and the results have generally supported the finding that high levels of NS predict increased substance use (Rose 1998; Gabel *et al.* 1999; Etter *et al.* 2003).

Efforts to explain these equivocal findings regarding the relationships among DRD4, NS and drinking behaviors have pointed toward a number of methodological and conceptual issues. Specifically, as noted by Laucht *et al.* (2007), the role of genetic factors in the etiology of alcoholism may vary at different stages of the trajectory between use initiation and dependence. This notion is consistent with the developmental behavioral-genetic perspective of alcoholism risk proposed by Rose (1998), in which the genetic and environmental influences on alcoholism risk vary across development. For example, the role of genetic factors in alcohol use initiation may be largely negligible but once alcohol use is initiated, differences in quantity and frequency of drinking appear to be strongly influenced by genetic factors (Rose 1998). Likewise, a twin study of personality traits in later adulthood has suggested that NS, but not other dimensions of personality, declined in total phenotypic variance across age cohorts (Heiman *et al.* 2003). Additionally, conflicting results highlight the need to carefully consider the phenotypes under study, given that more refined phenotypic measures, and endophenotypes, may increase sensitivity to detect genetic influences on behavior (e.g. Gottesman & Gould 2003; Hines *et al.* 2005). This is especially important when considering that the relative effect of single genetic polymorphisms on complex behaviors, such as alcohol use and abuse, is likely to be small in magnitude (Heath & Phil 1995).

In summary, there have been a number of studies examining associations among the DRD4 VNTR genotype, NS and drinking phenotypes. Yet, very few studies have integrated these converging lines of research. In one such study, however, Laucht *et al.* (2007) found that NS mediated the association between the DRD4 VNTR genotype and heavy drinking in male adolescents from a high-risk community sample. This study seeks to replicate and extend the findings of Laucht *et al.* (2007) by addressing the following objectives: (1) to examine the interrelationships among DRD4, NS and drinking behaviors in order to test the direct effects of DRD4 genotype on NS and drinking behaviors; and (2) to test a mediational model in which NS mediates the relationship between genotype and drinking behaviors using a heavy-drinking mixed sex college sample. From a developmental perspective, this may be an opportune time to assess the relationships among genotype, personality traits and drinking as the incidence of both alcohol use and NS behaviors has been shown to peak in early adulthood (e.g. Wechsler *et al.* 1994; Clements 1999; Heiman *et al.* 2003) and the frequency and quantity of alcohol use appears to be under considerable genetic influence at this stage in development (Rose 1998). Consistent with the recent findings of Laucht *et al.* (2007), we hypothesize that the relationship between genotype and alcohol use will be mediated by NS.

MATERIALS AND METHODS

Participants

In total, 101 men and women (44 females) were recruited from a college campus through flyers and announcements in the weekly email bulletin. A total of 90 participants (50 males and 40 females) provided complete data and were included in this study (10 participants did not complete the TPQ-NS and one had missing genetic data). The average age was 21.9 (SD = 1.5; range = 21–29) and 93% of the sample was Caucasian. Inclusion criteria were the following: (1) a score of 8 or higher on the Alcohol Use Disorders Identification Test (Allen *et al.* 1997), indicating a hazardous drinking pattern; and (2) self-reported drinking frequency of three or more drinks (two for women) at

least twice per week. Although there was no exclusionary upper limit of alcohol use for this study, participants who were currently trying to quit drinking or had a history of treatment for a drinking problem were excluded and offered treatment referrals.

Procedure

The study protocol was approved by the Human Research Committee at the University of Colorado and all participants provided written informed consent after receiving a full explanation of the study. Eligibility was determined through telephone interviews and eligible participants were invited to the laboratory for a testing session. Upon arrival at the lab, participants provided informed consent, a saliva sample for DNA analyses, and completed a series of self-report measures of demographics, personality and drinking behavior. A subset of participants ($n = 38$) was selected based on genotype for the μ-opioid receptor gene (OPRM1) to complete an alcohol challenge session and the results of the experimental study with this genetically selected subsample are presented elsewhere (Ray & Hutchison 2004).

Behavioral measures

Alcohol use

Alcohol use was evaluated with a variation of the measure used by White & Labouvie (1989). The instructions defined one alcoholic drink as 'one beer, one glass of wine, or one serving of hard liquor either by itself or in a mixed drink'. Two items asked 'In the last 12 months, (1) how often did you consume at least one alcoholic drink?' (answered on a 9-point scale ranging from 'never' to 'every day'), and (2) 'how many drinks did you usually have at one time?' (answered on a 10-point scale ranging from 'none' to 'more than 20 drinks'). Participants in this study drank an average of 4.9 (SD = 2.2) standard drinks per drinking episode and reported an average drinking frequency of slightly over twice weekly. Quantity and frequency items were standardized and averaged to form an alcohol use index, termed DRINK. Participants also reported the maximum number of drinks consumed in a single drinking episode during the past year (M = 13.9, SD = 5.9) and this drinking variable was termed MAX.

Rutgers Alcohol Problem Index (RAPI)

The RAPI was used to assess alcohol-related problems. This scale consists of 23 items examining the impact of alcohol on social and health functioning over the past year. In this sample, the average score on the RAPI was 22.2 (SD = 15.5). The RAPI has high reliability and validity (White & Labouvie 1989) and the observed Cronbach's α in this study was 0.93.

Tridimensional Personality Questionnaire, Novelty Seeking Scale (TPQ-NS)

The Novelty Seeking Scale of the TPQ (Cloninger *et al.* 1993) was used in this study. NS represents one of the four higher-order personality dimensions as defined by Cloninger's theory and consists of the sum of the following subscales: exploratory excitability versus stoic rigidity (NS1), impulsiveness versus reflection (NS2), extravagance versus reserve (NS3) and disorderliness versus regimentation (NS4). The TPQ-NS consists of 35 True/False items, such as 'I do things spontaneously' and 'I usually think about all of the facts in detail before I make a decision' (reverse scored). The average score on the TPQ-NS in this sample was 21.6 (SD = 5.2, range = 8–32) and the observed Cronbach's α was 0.76.

DNA analyses

DNA was collected following published procedures (see Freeman *et al.* 1997; Walker *et al.* 1999). Participants were asked to swab their cheeks with three cotton swabs, followed by a rinse of the mouth with tap water. Genomic DNA was isolated from buccal cells using a modification of published procedures (Lench, Stanier & Williamson 1988; Spitz *et al.* 1996). The 48 bp VNTR in the DRD4 was assayed using previously reported methods (Hutchison *et al.* 2002). The primer sequences used are forward, 5′-AGGACCCTCATGGCCTTG-3′ (fluorescently labeled), and reverse, 5′-GCGACTACGTGGTCTACTCG-3′ (Litcher *et al.* 1993). Alleles were visualized using capillary electrophoresis. Based on previous molecular work suggesting that the 7 repeat allele may confer a functional difference in D_4 receptors (Asghari *et al.* 1995) and molecular work demonstrating that the 7 repeat allele is quite distinct from the 2–6 repeat alleles and likely originated as a rare mutational event that became more frequent as a result of positive selection (Ding *et al.* 2002), participants were classified as DRD4 L (i.e. homozygous or heterozygous for an allele ≥7 repeats; S/L or L/L, coded '1'; $n = 29$) or as DRD4 S (i.e. both alleles <7 repeats; S/S, coded '0'; $n = 61$). For quality assurance purposes in the event of ambiguity in the genotyping, the assay is run in duplicate or triplicate in order to verify the results. The observed genotype frequencies (i.e. approximately 30% of participants were carriers of the L allele) are consistent with previous studies of primarily Caucasian and unselected samples (e.g. Hutchison *et al.* 2002, 2003; Munafò *et al.* 2008).

Data analysis

Pearson Product Moment correlations were calculated to examine the interrelationships among the variables of interest. Mediational analyses were conducted using a structural equational modeling (SEM) framework. The

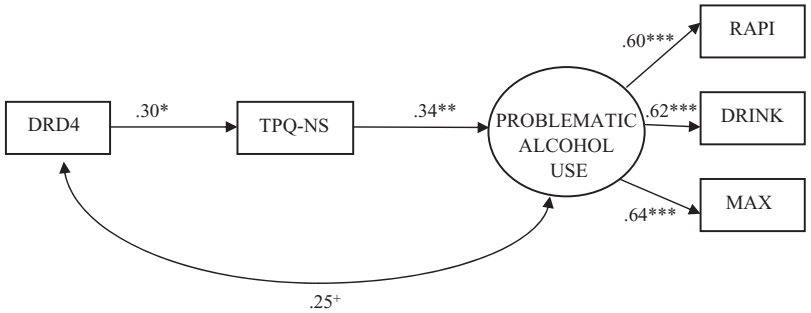

Figure 1 Model of problematic alcohol use among hazardous college drinkers, including mediational role of novelty seeking. Coefficients are standardized path coefficients. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{+}P = 0.07$. DRD4 = dopamine D_4 receptor; RAPI = Rutgers Alcohol Problem Index; TPQ-NS = Tridimensional Personality Questionnaire, Novelty Seeking Scale.

hypothesized model (see Fig. 1) examined NS as a mediator of the effects of DRD4 VNTR genotype on alcohol use. Problematic alcohol use was conceptualized a latent variable with three indicators: (1) RAPI score for the past 12 months; (2) standardized quantity and frequency of drinking over the past 12 months (DRINK); and (3) highest number of drinks in a single drinking episode over the past 12 months (MAX). Modeling analyses were conducted using the EQS Version 6.1 for Windows SEM program (Bentler 1995). Model fit was assessed with the comparative fit index (CFI, Bentler 1990) and the root mean square error of approximation (RMSEA; Browne & Cudeck 1993). Both the CFI and the RMSEA are sensitive to model misspecification and are minimally affected by sample size (Hu & Bentler 1995). The CFI ranges from 0 to 1, with 0.90 indicating acceptable fit (Bentler 1990). The RMSEA ranges from 0 to 8, where fit values less than 0.05 indicating close fit and values less than 0.10 indicating reasonable fit (Steiger 1990). In addition, model fit was assessed using the standardized root mean square residual (SRMR), which represents the standardized difference between the observed covariance and predicted covariance, with a value of zero indicating perfect fit. This measure tends to be smaller as sample size increases and as the number of model parameters increases. A value less than 0.08 indicates a good fit (Hu & Bentler 1999).

RESULTS

Dopamine D_4 receptor groups differed significantly with regard to gender, such that 20% ($n = 8$) of women had at least one copy of the DRD4-L allele, as compared with 40% ($n = 20$) of men ($\chi^2 = 4.43$, $P < 0.05$). DRD4 groups, however, did not differ with regard to age or ethnicity ($P > 0.10$); therefore, it is highly unlikely that population stratification confounded the analyses presented herein. Correlations among the study variables are presented in Table 1.

The estimated mediational model with standardized path coefficients is presented in Fig. 1. The model was found to provide an adequate fit of the data, $\chi^2(4, n = 89) = 1.66$, $P = 0.80$, CFI = 1.00, RMSEA = 0.00, 90% CI [0.00 to 0.10], SRMR = 0.02. This model accounted for 23% of the variance in problematic alcohol use. In the proposed model, DRD4 VNTR genotype was found to be significantly associated with NS, such that carriers of the long allele scored higher on a trait measure of NS (i.e. TPQ-NS), $B = 0.30$, $P < 0.05$. NS, in turn, was significantly and positively associated with problematic alcohol use, such that individuals reporting higher trait levels of NS reported higher problematic alcohol use, $B = 0.34$, $P < 0.01$. The following strategy was used to test mediation. First, we estimated a model with only the predictor (i.e. DRD4 VNTR genotype) and outcome variable (i.e. problematic alcohol use) included to assess the direct effect exclusive of any mediator variables. Results indicated a significant direct relationship between the predictor and the criterion, $B = 0.274$, $P < 0.05$. Second, we included a direct path from the DRD4 VNTR to problematic alcohol use in the context of the mediational model to determine whether the significant bivariate relationship between predictor and outcome had been reduced to nonsignificance. As can be seen in Fig. 1, the direct effect from DRD4 VNTR genotype to alcohol use was reduced to $B = 0.245$, $P = 0.07$, and the implementation of the Sobel test via EQS suggested that the indirect effect from DRD4 VNTR genotype to alcohol use through NS showed a trend toward significance, $est = 0.274$, $P = 0.06$. Both of these findings, i.e. a reduction in the size of the direct effect from predictor to criterion in the presence of the mediator, and a close to significant test of the indirect effect, are suggestive of at least partial mediation.

Table 1 Correlations among model variables.

	DRINK	*MAX*	*RAPI*	*TPQ-NS*	*DRD4*	*Gender*
DRINK	1.0					
MAX	0.42***	1.0				
RAPI	0.37***	0.38***	1.0			
TPQ-NS	0.21*	0.24*	0.32**	1.0		
DRD4	0.24*	0.24*	0.18	0.30**	1.0	
Gender	0.22*	0.40***	0.10	0.13	0.22*	1.0

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. DRD4 = dopamine D_4 receptor; RAPI = Rutgers Alcohol Problem Index; TPQ-NS = Tridimensional Personality Questionnaire, Novelty Seeking Scale.

Given that sex was significantly associated with the variables in the hypothesized mediational model and in a

recent study (Laucht *et al.* 2007), we estimated the model in Fig. 1 as a cross-groups analysis in EQS to test for moderation of any model relationships by sex. In a cross-group analysis in EQS, Lagrange multiplier tests (LM tests) are calculated for each parameter constrained to be equal across groups (Bentler 1995). This approach was chosen over testing separate models for males and females given sample size limitations and resulting lower statistical power. This approach allows us to capitalize on the standard errors in the full sample in our tests of cross-group differences, thus reducing the probability of type II error that would be incurred from conducting two separate tests. A significant LM test suggests a parameter on which the two groups differ. A1 d.f. chi-squared test of change ($\chi^2\Delta$) in model fit when a path is constrained to be equal across groups versus when it is allowed to differ across groups provides a test of the equality of a path in the two groups. A significant $\chi^2\Delta$ suggests that the path is indeed different across groups. Results revealed that none of the LM tests were significant, suggesting that the relationships among the variables were not significantly different for men versus women. Moreover, the cross-group model constraining all structural paths and the loadings on the alcohol latent factor to be equal across sex provided an adequate fit to the data, $\chi^2(13, n = 89) = 7.35$, $P = 0.88$; CFI = 1.00; RMSEA = 0.00, 90% CI [0.00 to 0.07]; SRMR = 0.06. Together, these results provide evidence of partial mediation. Specifically, the effects of DRD4 VNTR genotype on problematic alcohol use in this heavy-drinking college sample are partially accounted for by NS personality traits, and these effects are not moderated by sex.

DISCUSSION

The objective of the present study was to integrate convergent lines of research on the associations among the DRD4 VNTR genotype, NS and drinking behaviors. To that end, we examined a multivariate mediational model in which the effect of the DRD4 VNTR genotype on problematic alcohol use was mediated by NS. Results of SEM-based analyses provided support for partial mediation, whereby including NS in the model reduced the significance of the bivariate relationship between the candidate gene and problematic alcohol use in a sample of heavy-drinking college students. These results replicate and extend those of Laucht *et al.* (2007) and indicate that the mediational effects of NS may be present among both males and females. Contrary to Laucht *et al.* (2007), these results suggest partial mediation of genotype effects on drinking behavior through NS, as opposed to full mediation. Moreover, in the present study, these effects were not moderated by gender, as reported by Laucht *et al.* (2007). Notable differences in sample characteristics, such as the fact that the current study focused on heavy-drinking college students whereas Laucht *et al.* (2007) studied a selected sample of at risk adolescents, may explain the differences in the level of mediation and the gender specificity of the effects.

As recently articulated by Dick, Rose & Kaprio (2006), one of the major challenges in the field of psychiatric genetics consists of delineating the risk specifically tied to particular genes across development and in conjunction with environmental risk factors. To that end, studies that take into account developmental factors and multiple theory-driven variables may ultimately lead to more complete models that are also more useful. The present study suggests that in a sample of heavy-drinking young adults, the DRD4 VNTR genotype is associated with problematic alcohol use and that these effects are partially accounted for by genotype effects on NS. These results may help elucidate the specific pathways of risk associated with genetic influences on alcohol misuse. Importantly, the integration of statistical and behavioral methods provides a promising framework to study risk pathways for psychiatric phenotypes.

These findings highlight the importance of more accurately characterizing phenotypes, which in turn could help clarify inconsistent findings. Specifically, more refined phenotypic measures, such as endophenotypes, may increase sensitivity to detect genetic influences on behavior (e.g. Gottesman & Gould 2003; Hines *et al.* 2005). This is especially important when considering that the relative effect of single genetic polymorphisms on complex behaviors, such as alcohol use and abuse, is likely to be small in magnitude (Heath & Phil 1995). In terms of characterizing drinking phenotypes, the present study treated problematic alcohol use as a latent variable with three indicators, namely alcohol-related problems, quantity and frequency of drinking, and maximum number of drinks per episode. Similar approaches may be useful in behavior genetics studies of complex disorders as a means of increasing statistical power to detect genotype effects (by decreasing measurement error associated with the phenotype), decreasing multiple comparisons and ultimately producing more consistent findings.

A number of aspects of the current findings warrant further discussion and raise questions for future research. First, at this point, the underlying molecular processes that account for these effects are unclear. Based on the existing literature, two alternative explanations for these differences exist. From a molecular standpoint, D_4 receptors are widely distributed in the brain, but with notable localization in the ascending corticomesolimbic dopamine axis (Wedzony *et al.* 2000; Berger *et al.* 2001; Svingos, Periasamy & Pickel 2001), running from the ventral tegmental area to the nucleus accumbens and projecting into

the prefrontal cortex. In the associated regions, as D_2-like receptors, activation of D_4 receptors serve to inhibit the accumulation of intracellular cAMP. In turn, recent findings suggest that greater cAMP in limbic regions, specifically the nucleus accumbens, results in greater motivation for dopaminergic rewards (Knapp *et al.* 2001; Lynch & Taylor 2005; Choi *et al.* 2006). Therefore, given that the D4.7 variant may be functionally less sensitive (i.e. permits greater cAMP accumulation), it is plausible that greater tonic levels of limbic cAMP may account for both greater NS and alcohol use in D4.7 carriers. Alternatively, a recent neuroimaging study found that the D4.7 allele is associated with greater activation in the prefrontal cortex in response to alcohol cues (McClernon *et al.* 2007), suggesting that D4.7 carriers may engage in greater reward processing through cognitive mechanisms. In the context of the current study, the two possibilities of DRD4 VNTR-mediated alterations to limbic or frontal dopaminergic neurotransmission must remain speculative and await further research.

The second area of the study that warrants further discussion pertains to the behavioral findings. As noted earlier, the construct of NS is multifarious, including aspects of impulsivity, excitability and extravagance (Cloninger *et al.* 1993). As such, the subcomponents of NS may represent even narrower behavioral phenotypes and it is unclear which subcomponent(s) are most influenced by DRD4 VNTR genotype. Investigating these fine-grained relationships may further clarify the functional influences of molecular variation at the DRD4 locus. Moreover, consistent with the partial mediation effects, clarifying the connection between DRD4 VNTR and alcohol use, beyond the influence of NS, remains a priority for future studies.

These results should be interpreted in the context of the study's strengths and limitations. Study strengths include a theory-driven multivariate model of genetic effects and the use of advanced statistical methods. Limitations include the relatively small sample comprised of heavy-drinking college students. The small sample combined with the unequal number of men and women limited our ability to detect significant cross-groups differences (Kaplan & George 1995), and thus we caution that because of the possibility of increased type II error, future studies are necessary to fully understand the moderating influence of sex on these relationships. This was a cross-sectional study and longitudinal approaches seem warranted. Limitations notwithstanding, these findings advance the understanding of DRD4 VNTR genotype in relation to alcohol use phenotypes and personality traits in heavy-drinking young adults. Future studies that can further dissect these interrelationships will be essential to fully characterize the behavioral effects of variation at the DRD4 locus.

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